

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Docket No: Q86326
Hiroshi FUKUSHTMA, et al.
Serial No. 10/525,748 Group Art Unit: 1626
Filed: October 11, 2005 Examiner: Karen CHENG
For: BENZENESULFONATE SALTS OF 4-FLUORO-2-CYANOPYRROLIDINE
DERIVATIVES

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
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Sir:

I, Hiroshi Fukushima, a Japanese citizen, residing at
1643-3, Suka, Miyashiro-machi, Minamisaitama-gun, Saitama,
345-0831, Japan, do hereby solemnly and sincerely declare that:

I graduated from Chiba University in 1983 and obtained a
master's degree from the same University in 1985;

I am working for the Pharmaceuticals Laboratory of Taisho
Pharmaceutical Co., Ltd., the Assignee of the above-identified
application;

I am one of the co-inventors of the above-identified
application and am familiar with the disclosure and the claims
of said application; and

in order to prove that the benzenesulfonate salt of

(2S,4S)-2-cyano-4-fluoro-1-[(2-hydroxy-1,1-dimethyl)ethyl-aminol]acetylpyrrolidine according to the present invention (hereinafter referred to as "the benzenesulfonate salt of the present invention") has an advantageous effect in that it is particularly excellent in the crystallinity required for pharmaceutical preparations when compared to other salts. I conducted the following experiment to compare the crystallinity between the benzenesulfonate salt of the present invention and the corresponding tosylate salt.

The reason why the tosylate salt was selected as a control for comparison purposes is because a tosylate salt is structurally most similar to and also more commonly used as a pharmaceutical preparation than a benzenesulfonate salt.

To this end, standard crystallization procedures were used to study the easiness of salt preparation. Likewise, thermal analysis and powder X-ray diffractometry were used to study the presence or absence of crystal polymorphism.

Experiment

(1) Salt preparation

Benzenesulfonate salt

(2S,4S)-2-Cyano-4-fluoro-1-[(2-hydroxy-1,1-dimethyl)-ethylamino]acetylpyrrolidine (150 mg) was suspended in methanol (880 μ L). To this suspension, a solution of benzenesulfonic acid (114 mg, 1.05 equivalents) in methanol (140 μ L) was added dropwise under ice cooling and stirred for 15 minutes. Upon dropwise addition of the benzenesulfonic acid solution, the free form was rapidly dissolved and a solid crystal of its salt was then

precipitated from the solution. To this suspension, additional methanol (200 μ L) was added. After addition of isopropyl ether (5.0 mL), the suspension was stirred at room temperature for one hour. The precipitated crystal was collected by suction filtration and then dried in a desiccator under reduced pressure for 7 hours while heating at 65°C.

Tosylate salt

(2S,4S)-2-Cyano-4-fluoro-1-[(2-hydroxy-1,1-dimethyl)-ethylamino]acetylpyrrolidine (150 mg) was suspended in methanol (840 μ L). To this suspension, a solution of tosylic acid (123 mg, 1.05 equivalents) in methanol (160 μ L) was added dropwise under ice cooling. Immediately after addition of the tosylic acid solution, a large amount of crystal was newly precipitated from methanol before the free form was completely dissolved. When additional methanol (600 μ L) was added, the crystal was no longer dissolved. After addition of isopropyl ether (3.0 mL), the suspension was stirred at room temperature. The precipitated crystal was collected by suction filtration and then dried in a desiccator under reduced pressure for 7 hours while heating at 65°C.

Discussion of the results

The tosylate salt had too good a crystallinity and started to crystallize before the free form was completely dissolved, so that it was crystallized in a state incorporating impurities including the free form. Thus, due to having too good a crystallinity, the tosylate salt was likely to incorporate impurities and was difficult to obtain

as a crystal of high purity.

In contrast, in the case of the benzenesulfonate salt of the present invention, when a solution of benzenesulfonic acid in methanol was added dropwise to a suspension of the free form in methanol, the free form was rapidly dissolved and a solid crystal was then precipitated. Thus, the benzenesulfonate salt of the present invention was obtained as a crystal of high purity without contamination with the free form.

(2) Study on the presence of crystal polymorphism (thermal analysis (TG/DTA) and powder X-ray analysis)

Thermal analysis (TG/DTA)

I. To study the presence or absence of crystal polymorphism, the crystals obtained through the above standard procedures were used in thermal analysis as shown below for comparison between the benzenesulfonate salt of the present invention and the corresponding tosylate salt.

Each salt obtained as described above was taken in an amount of about 2 mg and measured using Thermoplus TG8120 (Rigaku Corporation) over the range of 30°C to 350°C (rate of temperature rise: 10°C/minute).

TG/DTA charts are shown in Figure 1 (benzenesulfonate salt) and Figure 2 (tosylate salt) attached hereto.

The benzenesulfonate salt of the present invention showed a single melting/endothermic peak. This gives no indication of polymorphism for the benzenesulfonate salt of the present invention.

The tosylate salt showed a peak which was observed for

the free form, suggesting that the crystal of tosylate salt was contaminated with the free form. The tosylate salt also showed some other peaks; this suggests the presence of polymorphism for the tosylate salt.

II. The presence of crystal polymorphism was further studied for the benzenesulfonate salt of the present invention when crystallized from different solvent systems other than the above methanol-isopropyl ether system.

Single solvent system: The salt (200 mg) was dissolved in a solvent (ethanol or acetonitrile) by heating and allowed to stand overnight at room temperature.

Double solvent system: The salt (200 mg) was dissolved in DMF by heating. To this solution, toluene was added at room temperature and stirred for about one hour.

The salts obtained from the above solvent systems were each taken in an amount of about 2 mg and measured using Thermoplus TG8120 (Rigaku Corporation) over the range of 30°C to 350°C (rate of temperature rise: 10°C/minute).

As a result, in either case, the benzenesulfonate salt of the present invention produced the same results (little loss in weight and a single melting/endothermic peak) as in the case of the crystal form obtained by crystallization from methanol-isopropyl ether shown in I above (Figure 1). This gives no indication of polymorphism.

Powder X-ray diffractometry

The benzenesulfonate salt of the present invention was studied by powder X-ray diffractometry using the crystal obtained by crystallization from methanol-isopropyl ether

shown in I above and the crystals obtained by crystallization from the solvent systems shown in II above other than the methanol-isopropyl ether system.

Each sample was filled into a glass holder and measured using RAD-C (Rigaku Corporation) under the following conditions.

Target: Cu, Scanning Speed: 4°/min, Voltage: 40 kV, Current: 30 mA, 2 θ : 3° to 40°, Slit DS: 1°, RS: 0.15 mm, SS: 1°

As a result, the benzenesulfonate salt of the present invention showed the same powder X-ray diffraction pattern in all cases. Figure 3 shows, as a representative, the powder X-ray diffraction pattern of the crystal obtained by crystallization from methanol-isopropyl ether. These results indicate that the benzenesulfonate salt of the present invention takes the same crystal form when crystallized from all of these solvent systems, and hence give no indication of polymorphism.

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 3rd day of June, 2007.

Hiroshi Fukushima
Hiroshi Fukushima

Fig. 1

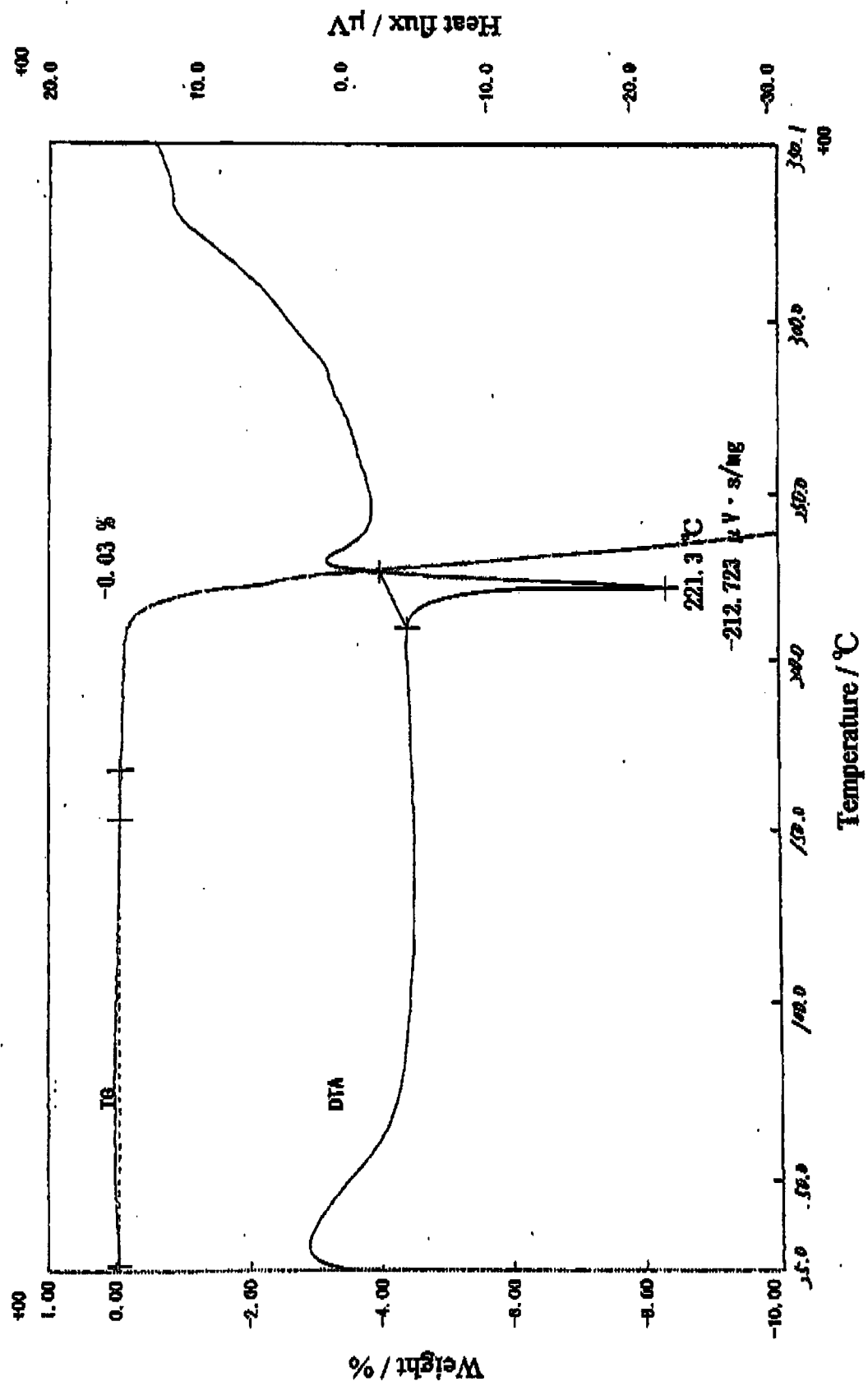


Fig. 2

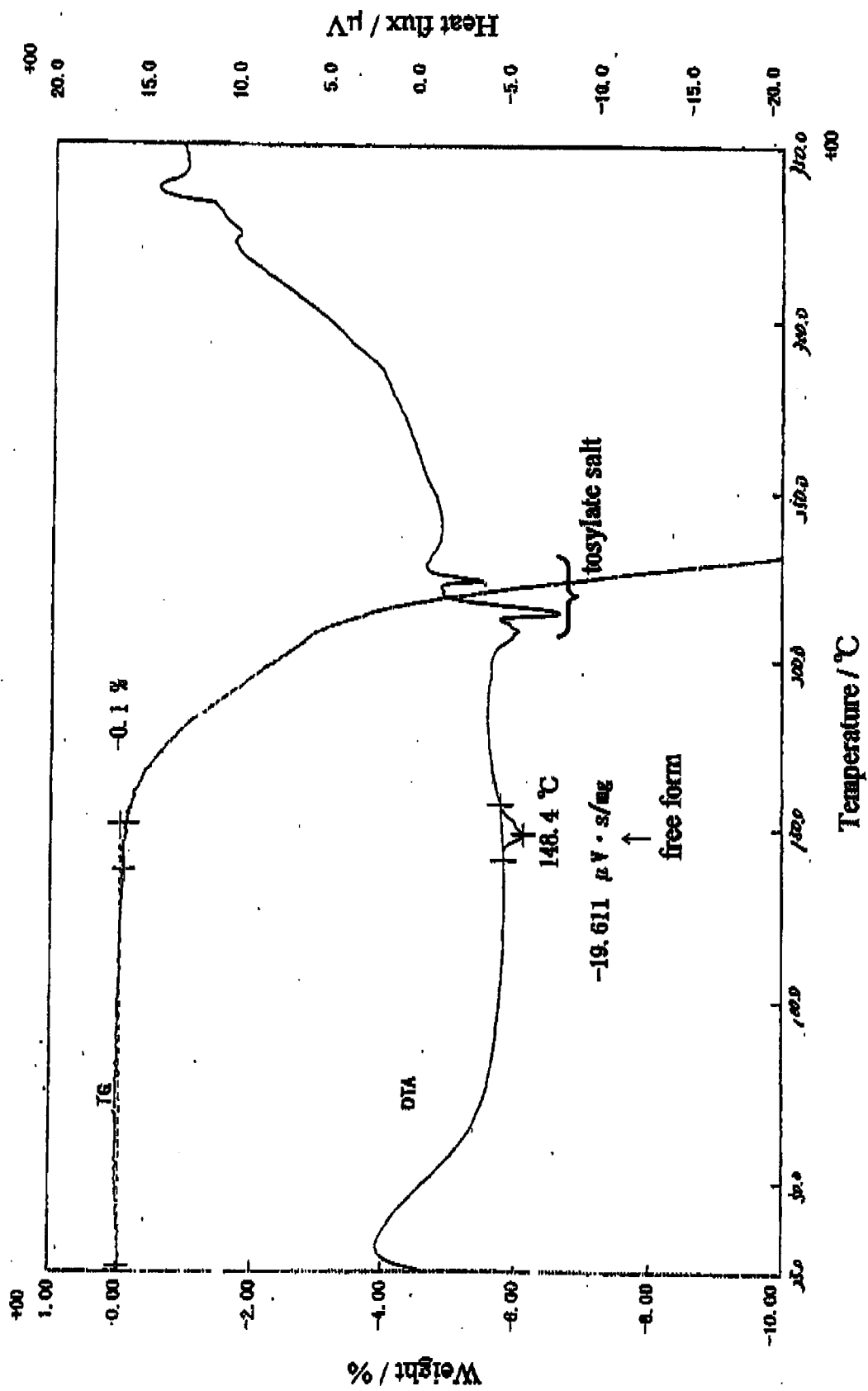


Fig. 3

